



Supplemental Fig. 1. Immunofluorescence detection of 3 β -HSD protein in cultured Leydig cells. Representative images for 3 β -HSD (pink) in Leydig cells. (A) The negative control showed no background labeling. (B) Cells were stained with 3 β -HSD antibody (sc-30820, Santa Cruz Biotechnology, Dalla, TX, USA) (1:100) and counterstained with DAPI to confirm nuclear status (blue). Leydig cells were cultured on glass coverslips, followed by fixation in 4% buffered formaldehyde. Cells were permeabilized by treatment with 0.3% Triton X-100 in PBS for 5 min and washed with PBS. The cells were blocked with 10% normal donkey serum in PBS at room temperature for 30 min and then incubated with goat polyclonal 3 β -HSD antibody (sc-30820, Santa Cruz Biotechnology, Dallas, TX, USA) diluted 1:100 in PBS containing 1% BSA overnight at 4°C. Cells were then washed with PBS and incubated with 1:200 donkey anti-goat IgG (Alexa® 647, ab150131, abcam, Cambridge, MA, USA) for 1 h at 37°C. They were washed again with buffer before counterstaining nuclear DNA with 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Inc., Burlingame, CA, USA). Cells were observed with a fluorescence microscope (Leica, Heidelberg, Germany). To rule out nonspecific staining, the first antibody was replaced with normal non-immune goat serum as a negative control. Bar = 50 μ m.